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RESEARCH ARTICLE

Use of Pupil Size to Determine the Effect of Electromagnetic Acupuncture on Activation Level of the Autonomic Nervous System

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Abstract
Magnetic fields are widely considered as a method of treatment to increase the therapeutic effect when applied to acupoints. Hence, this study proposes a new method which creates significant stimulation of acupoints by using weak magnetic fields. We conducted this experiment in order to confirm the effect on the activation level of the autonomic nervous system by measuring pupil sizes in cases of stimulation by using manual acupuncture and electromagnetic acupuncture (EMA) at BL15. We selected 30 Hz of biphasic wave form with 570.1 Gauss. To confirm the biopotential by the magnetic flux density occurring in EMA that affected the activation of the autonomic nervous system, we observed the biopotential induced at the upper and the mid left and right trapezius. We observed a significant decrease in pupil size only in the EMA group ($p < 0.05$), thus confirming that EMA decreased the pupil size through activation of the parasympathetic nerve in the autonomic nervous system. Moreover, we confirmed that the amplitude of the biopotential which was caused by 570.1 Gauss was higher than $\pm 20\ \mu A$. Thus, we can conclude that EMA treatment successfully activates the parasympathetic nerve in the autonomic nervous system by inducing a biotransformation by the induced biopotential.

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1. Introduction

Chinese medicine treats various lesions by making meridian's Qi flow smoothly by performing manual acupuncture. In other words, it is believed in Chinese medicine that diseases are evoked due to imbalance in the body that is caused by Qi stagnation. In a healthy body, Qi flows smoothly throughout the body through meridians. To cure the imbalance, manual acupuncture is performed to release stagnant Qi [1]. Therefore, various researchers investigated the objective treatment mechanism of manual acupuncture, and reported varied research results regarding activation of nerves or internal secretion, and effects on cerebrospinal fluid [2—4]. Moreover, the effectiveness of manual acupuncture treatment was also reported for osteoporosis [5], hyperlipidemia [6], and pain relief [7]. However, patients avoid manual acupuncture because of its invasiveness. Thus, patients are recommended to receive the transcutaneous electrical nerve stimulation (TENS), which only stimulate local parts such as acupoints [8,9].

Numerous studies reported the possibility of using weak magnetic fields as an alternate treatment method [10,11]. Magnetic fields are applied to treat various illnesses such as fractures, pain, arteriosclerosis, and Parkinson's disease, and the method is proven to be most effective in treating neurologic diseases [12—14]. Thus, it is possible to induce curative influences on various lesions using weak magnetic fields. However, weak magnetic fields do not create enough stimulation on an acupoint located deep beneath the subcutaneous tissue because the magnetic flux density generated decreases geometrically according to the distance.

Hence, it is impossible to stimulate an acupoint located deep in the muscular tissue, not in subcutaneous tissue. For this reason, many clinical treatments have been performed with electro-acupuncture (EA) and not with invasive treatment methods, and it was reported that it is more effective than manual acupuncture due to additional stimuli [15—17]. However, EA is still used by most of the clinics because noninvasive methods are limited in terms of how deep they can penetrate. However, EA has a critical problem in that it requires two electrodes because of a potential difference in the current flow. Thus, it is necessary to suggest a new method that can stimulate vertically only at acupoints in order to penetrate deeply into the tissue and generate bioelectric currents without another electrode and external current supply. Thus, this paper proposes a new method which creates significant stimulation on acupoints with weak magnetic fields in similar forms of EA. As solenoid forms of magnetic fields generate magnetic flux from the core, it is possible to stimulate deeper parts rather than simply stimulating an acupoint when electromagnetic acupuncture (EMA) with a manual acupuncture needle for substitution for core is used. In addition, it could increase the therapeutic effect by inducing the reaction inside the body with both manual acupuncture and magnetic fields as reported in the past. Moreover, after the magnetic fields penetrate into an electric conductor such as the body, bioelectric currents can generate eddy currents in the tissue and impact various tissues such as the nerve or muscular tissue, etc., without another electrode.

In order to objectively observe the degrees reflected on the activation of autonomic nerve systems by manual acupuncture on an acupoint, electroencephalogram (EEG), heart rate variability (HRV), skin conductance response (SCR), and pupil size variability (PSV) were observed [18,19]. Two soft muscles that control the size of pupils are directly influenced by the sympathetic nerve of the autonomic nerve system and cause antagonism of the parasympathetic nerves. Therefore, PSV is a better method of observing activation information of the autonomic nerve system. Accordingly, research regarding the interrelationship of activation of autonomic nerve systems from PSV have been conducted previously and identified varied results such as activation of the parasympathetic nerve by drowsiness or fatigue, activation of the parasympathetic nerve by contraction mechanisms induced by cold stimulation, etc. [20—24].

Accordingly, a coil where a manual acupuncture needle can be inserted was produced to observe and analyze the effect of manual acupuncture and EMA on the autonomic nerve system. Also, an eight-channel system was designed in order to stimulate many acupoints at once. In addition, the range of pulsed electromagnetic fields (PEMFs) and extremely low frequency (ELF $\leq$ 300 Hz) were selected as they were proven to have therapeutic effects on the nerve system by making depolarization of adjacent nerves after creating eddy currents as they penetrate into the human body. A pupillometer was designed using an infrared camera to measure the pupil size precisely and sequentially. Then, the activation level of autonomic nerve system was evaluated by measuring the pupil size when performing manual acupuncture and EMA on bladder meridian about BL15.

Existing neurological research established that spinothalamic tract cells are hardly activated by an input on the skin creating stimulation in the heart and the lung. However, it is reported that they are stimulated significantly when the cells get additional input from muscles [25,26]. Therefore, we observed the induced biopotential by magnetic flux density occurring in EMA to study the effect on the muscle, and analyze the effect on the activation level of the autonomic nerve system.

2. Materials and methods

2.1. Selection on an acupoint

In the central nervous system, 12 pairs of cranial nerves, 31 pairs of spinal nerves and autonomic nervous systems, which control an endocrine gland of involuntary muscles and blood vessel systems, pass through the spinal cavity. T1—4 (thoracic vertebrae) affects cardiovascular and respiratory activity [27]. When the patient is in a stable condition, activation of the parasympathetic nerve and stabilization of the autonomic nerve increase the function of the cardiovascular system by relaxation of the body through overcoming stress and bathypnea (deep breath). Therefore, BL15 of bladder meridian was selected as it represents a heart according to meridian.

2.2. PEMFs system design

Fig. 1 shows the system and the coil of solenoid form with a diameter of 15 mm and length of 20 mm. In order to create high magnetic flux in the tip of the manual acupuncture needle, we designed a hole-shape coil with a diameter of 2 mm where a manual acupuncture needle can be inserted in the core of SM45 C with a length of 16 mm and a diameter of 5 mm. The coil was wound a total of 370 times using a wire, 0.35 mm in diameter. Also, we designed a wing-shaped electrode case for the solenoid coil so that the coil can be attached to an acupoint easily, and does not come into direct contact with the skin. The eight-channel system was operated with a touch screen input system to control functions such as channel activation, stimulation frequency, and timer. The level of magnetic flux density was varied depending on the current permitted to coil, so are a total of five levels of supplied current to coil to create magnetic flux intensity. It was designed to adjust the range of stimulation frequency from 1 Hz to 30 Hz. By controlling the direction of permitted current on the coil, it was possible to select the occurrence direction of magnetic flux density as either a monophasic wave or a biphasic wave. The coil has a safety function, which senses the high heat with the temperature sensor, as well as a safety circuit and emergency system stop function.

The stable manual acupuncture method regarding BL15 inserts the manual acupuncture needle in the direction of the spinal cord 1.52—2.42 cm deep with an angle of 45—60°. The maximum insertion depth is between 4.24 cm and 4.84 cm. The depth of manual acupuncture was selected as 1.5 cm considering the relationship between the solenoid firm of coil and the length of the manual acupuncture needle (stainless steel, diameter: 0.3 mm, length: 30 mm, DongBang Acupuncture, Ungcheon-eup, Boryeong-si, Korea) and 90 degrees of slope to attach the electrode case. Accordingly, the manual acupuncture needle was inserted and fixed so that it would come out of coil for 1.5 cm. Then the magnetic flux density generated from the manual acupuncture needle depending on stimulation mode and stimulation frequency was measured using a Teslameter (Model 460, 3Channel Gaussmeter, Lake Shore Crytronics, Inc., Westerville, Ohio, USA). In order to observe the decrease in magnetic flux density according to distance and confirm the range of effect, the magnetic flux density was measured at a certain distance between the Teslameter electrode and the tip of the manual acupuncture needle. Fig. 2 is the result of generated magnetic flux density in two stimulation modes after selecting a supply voltage to make maximum magnetic flux density. It was observed that the magnetic flux density increased according to the increase in stimulation frequency, and the biphasic wave form had a greater magnetic flux density than the monophasic wave form. Furthermore, we observed that the magnetic flux density was generated up to a distance of 3 cm away from the manual acupuncture needle as shown in Fig. 3. Based on the results, we selected 30 Hz of a biphasic wave form with 570.1 Gauss to stimulate BL15 with appropriate maximum magnetic flux density.

2.3. Pupillometer design

The image acquiring system was designed using infrared camera Logitech HD Pro Webcam C910, Yeouldo-dong, Seoul-si, Korean, infrared lens, and infrared filter. The image of a pupil was captured with the self-designed user

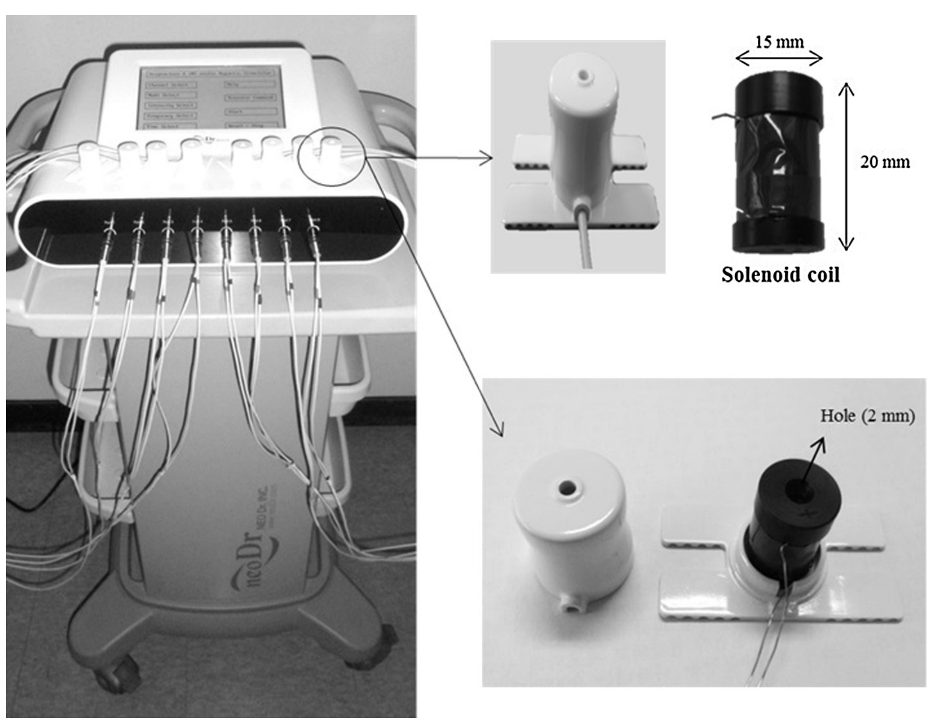


Figure 1 The solenoid coil for magnetization of manual acupuncture needle and eight channel PEMFs stimulator. PEMFs = pulsed electromagnetic fields.

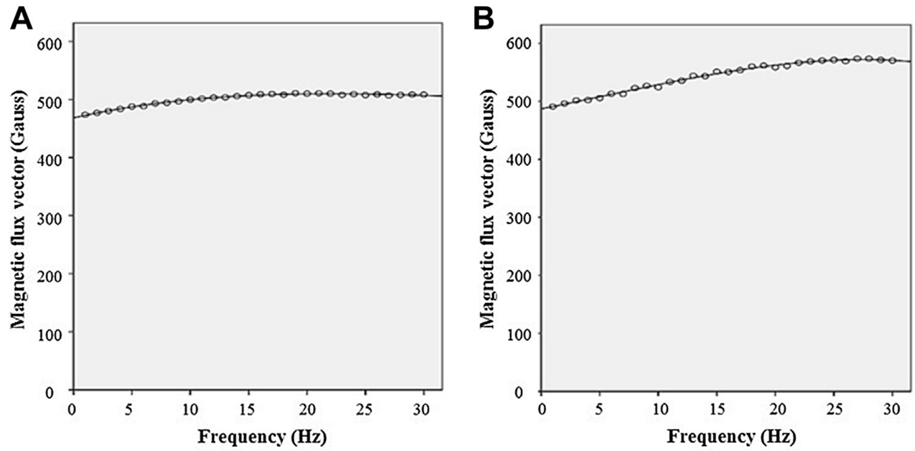


Figure 2 The magnetic flux density at (A) monophasic wave and (B) biphasic wave.

interface based on Visual Studio 2010 C# program, Redmond, Washington, America and DirectShow Library as in Fig. 4. After recording with 30 frames/second and 640 $\times$ 480 resolution, the pupil size data were collected by acquiring the frames, processing each frame with the same image processing shown in Fig. 5. In general, a pupil is shaped like an ellipse, so more precise pupil size data were obtained by using the curvature algorithm and ellipse fitting during image processing procedures rather than the circle fitting, which other previous studies have used [28—30].

The existing research reported that the size of a pupil in the supine position is significantly smaller than the size of a pupil in a sitting or standing position [29]. Supine positions activate parasympathetic nerves more than any other position and also makes the size of the pupil smaller. We selected the sitting position in order to exclude the effect in the pupil size caused by stability of the patient's supine position and to measure the change in the pupil size caused only by manual acupuncture or EMA. As shown in Fig. 6, a pretest was conducted to observe when changes in the size of the pupil occur most frequently as patients complain regarding the inconvenience of sitting in a fixed position. Ten participants stared at the circle with a diameter of 1 cm located 60 cm away from the participants in a seated position without any stimulation. After 3 minutes of stabilization, the size of the pupil was measured for 10 minutes. All data were normalized based on the average size of a pupil for 2 minutes. As a result of the contrast test of the one-way repeated measures analysis of variance (ANOVA), it was observed that the pupil size had a significant difference from 180 seconds ($p = 0.020$). Thus, we chose 200 seconds as the measurement time based on the results of the preceding research.

2.4. Participants and experimental procedure

Thirty males (age: 26 $\pm$ 2.6 years old, height: 175 $\pm$ 4.5 cm, weight: 71 $\pm$ 11.2 kg) who did not have a medical history in the department of ophthalmology, psychiatry, and cardiovascular diseases participated in this experiment voluntarily. We selected people who fully understood the contents of this experimentation and agreed to participate, and groups of 10 persons were allocated randomly into the nonstimulation group, the manual acupuncture group, and the EMA group. To minimize error, all participants were forbidden to exercise intensively one day prior to the experiment, ingest excessive food and caffeine, and smoke 2 hours prior to the experiment. The temperature in the experiment room was maintained at 25°C, and the experiment was conducted in a darkroom to minimize any errors caused by light sources from outside.

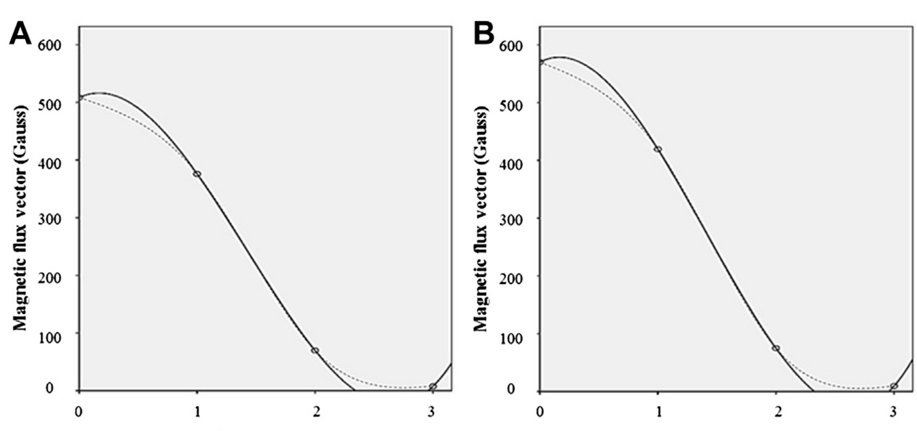


Figure 3 The magnetic flux density at (A) monophasic wave and (B) biphasic wave by distance (—: cubic curve, ——: spline curve).

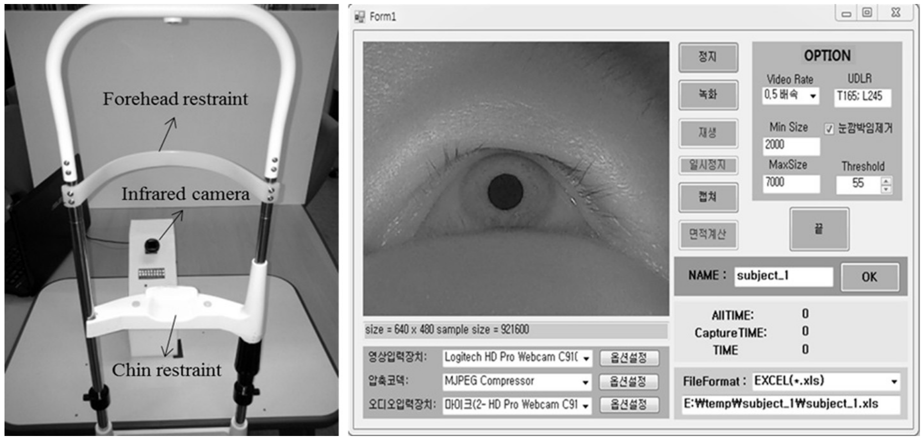


Figure 4 The designed pupillometer and image processing interface.

The size of the pupil was measured in the cube place of 60 cm × 60 cm in order to avoid the effect of the surrounding environment. All participants stared at the goal point which was 60 cm ahead of them and their left pupil was measured after keeping their head and chin fixed. All participants were stabilized for 3 minutes so that they could adapt to the experiment environment. Then, the size of the pupil was measured for 200 seconds. All groups received related stimulation for 200 seconds each.

2.5. Biopotential measurement

Ten participants attended the biopotential measurement experiment. As BL15 is located on the trapezius muscle, the upper and mid left and right trapezius muscles were selected. As shown in Fig. 7, the position of the electrodes was the same as the position of the electrodes for measuring the electromyogram (EMG) to confirm the biopotential generated at comprehensive trapezius muscles.

To measure the biopotential at the upper trapezius muscle, two active circular Ag/AgCl surface electrodes (Electrode number 272; diameter: 14 mm, interval distance: 18 mm, Noraxon Inc., USA) were placed on the upper trapezius, along the ridge of the shoulder. Two active electrodes were placed horizontally 2 cm apart. To measure biopotential at the middle trapezius muscle, we placed two active circular Ag/AgCl surface electrodes on the middle border of the spine of the scapula. A ground electrode was placed on the right head of the ulna. The skin where the electrode was to be placed was sterilized and the hair was plucked. The biopotential was filtered between 10 Hz and 500 Hz and pre-amplified as 1000 gain. The biopotential was converted to 12-bit processing with a sampling frequency of 1 kHz. The digitized biopotential was monitored and stored in a GUI with Noraxon Myoresearch XP software (Noraxon Inc., Scottsdale, Arizona, America). The biopotential measurement of both the upper and mid trapezius were recorded for 180 seconds. All participants maintained a steady state for 60 seconds and received the PEMFs stimulation between 60 seconds and 120 seconds. All participants maintained a steady position for 60 seconds.

2.6. Statistical analysis

The change of the pupil size was analyzed after applying normalization on the measured size of the pupil which was

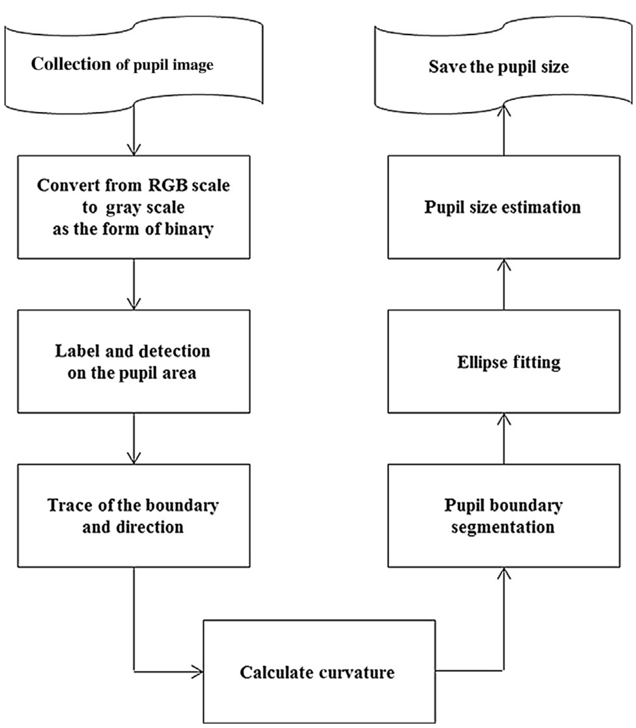


Figure 5 The algorithms for detecting pupil size.

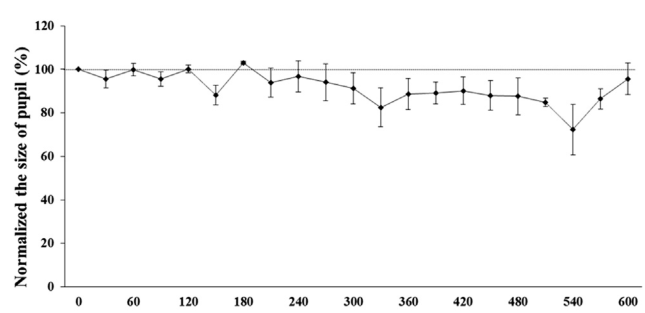


Figure 6 The changes in pupil size by time.

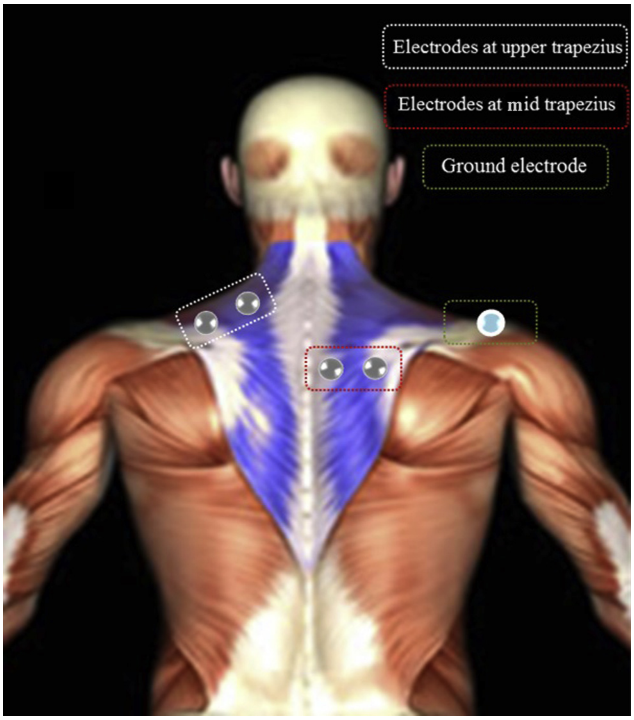


Figure 7 The position of electrodes for measuring biopotential.

measured for 200 seconds using stabilized average pupil size measured for 3 minutes as standard. The contrast test based on the one-way repeated measures ANOVA was conducted with the dependent variable (size of the pupil) on three groups. The significance level of the statistical analysis was set at $p < 0.05$. To obtain the significant differences in median frequency (MF) by time (prior to stimulation, during stimulation, and after stimulation), a one-way ANOVA and *post hoc* analyses were conducted.

3. Results

3.1. The changes in PSV

Fig. 8 illustrates the result of pupil size for 200 seconds of the nonstimulation group, the manual acupuncture group, and the EMA group. As shown in the Tables 1–3, the maximum pupil size of the nonstimulation group was 109.28 $\pm$ 11.90 (%) at 190 seconds, 86.89 $\pm$ 15.22 (%) at 180 seconds for the manual acupuncture group, and 71.04 $\pm$ 10.09 (%) at 200 seconds for the EMA group. As a result of the contrast test based on the one-way repeated measures ANOVA, the difference in pupil size was not statistically significant for the nonstimulation group and the manual acupuncture group ($p > 0.05$). However, we confirmed that the EMA group had a significant difference in the pupil size after 140 seconds ($p < 0.05$).

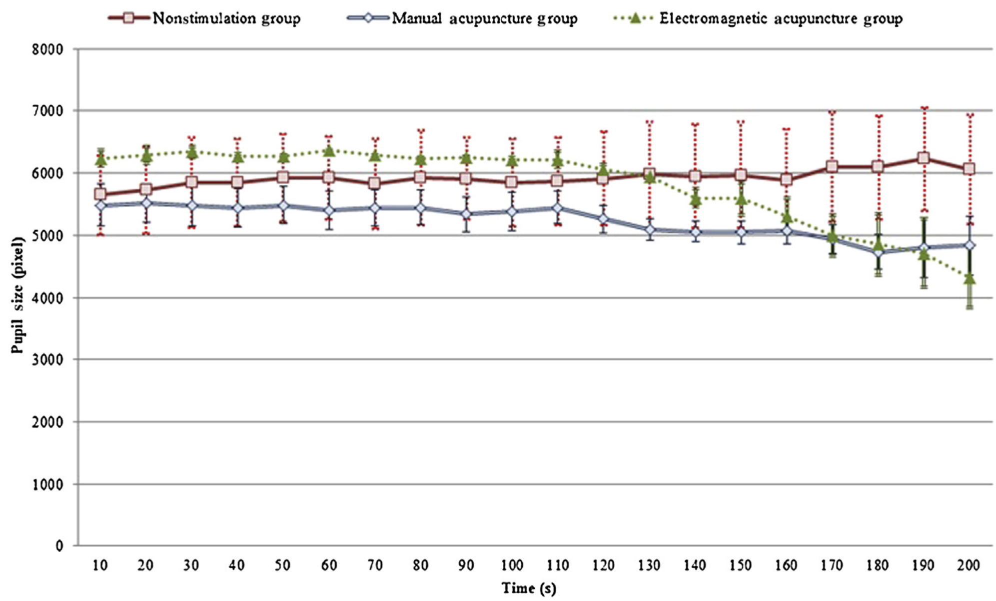
3.2. Results of the biopotential

Fig. 9 shows the results of biopotential induced by EMA in the left/right mid trapezius, and Fig. 10 shows the results in the left/right upper trapezius. We confirmed that biopotential was induced between 60 seconds and 120 seconds, except steady state among total measured data for 180 seconds. The biopotential was provoked by 52 uV in the left mid trapezius with a maximum of 23.79 $\pm$ 3.12 uV and minimum -20.43 ± 2.52 uV, and in the right mid trapezius with maximum 27.46 $\pm$ 1.9 uV and minimum -24.35 ± 2.94 uV. The maximum of 67.12 $\pm$ 12.34 uV and minimum of -56.67 uV $\pm$ 8.73 uV in the left upper trapezius, and maximum of 37.90 $\pm$ 4.5 uV and minimum of -49.77 ± 8.02 uV in the right upper trapezius were created. Both Fig. 9C and D and Fig. 10C and D represent biopotential created for 1 second when EMA was stimulated. We confirmed that biopotential was induced for 30 sets for 1 second identical to the EMA stimulation frequency. Fig. 11 shows the changes in the normalized median frequency (MF) obtained through the power spectrum analysis. There was a significant difference between median frequency prior to stimulation and during stimulation ($p < 0.05$). The median frequency was recovered to the level prior to stimulation ($p > 0.05$).

4. Discussion

Existing research reports that activation of sympathetic nerves and parasympathetic nerves is induced differently depending on where manual acupuncture is applied [31]. Numerous studies were conducted to analyze the effect of manual acupuncture of BL15 on the autonomic nerve system. Previous research reports that the effect of manual acupuncture of BL15 leads to the activation of parasympathetic nerves based on the result of the analysis of both HRV and pulse rate variability (PRV) [32]. Among autonomic nerve system limbs, the parasympathetic nerve limb can create atrial fibrillation in the atrium. Therefore, the result of the HRV when stimulated at BL15 by manual acupuncture is able to indicate the possibility of activation of the parasympathetic nerve. As previous studies have stated, we found BL15 an important point to affect the autonomic nerve system.

Power spectral analysis of HRV is a useful noninvasive method for the assessment of the autonomic nervous system. Three vital oscillatory components were identified as follows: the very-low-frequency (VLF) component (frequency bandwidth is still controversial), the low-frequency (LF) component (from 0.04 Hz to 0.15 Hz) and the high-frequency (HF) component (from 0.15 Hz to 0.4 Hz). The LF component is influenced by baroreflex sympathetic control of blood pressure. HF component and parasympathetic control of the heart rate are closely related [33,34]. The LHR is calculated as the ratio of LF component to HF component and is used to evaluate the sympathetic and parasympathetic activities of the autonomic nervous system [35,36]. The measurement time is divided into two. One is performed on the basis of 24 hour Holter recordings (long-term recordings) [37,38], and the other method needed a shorter period ranging from 0.5 minutes to 5 minutes for collecting data from 200 heartbeats to 500 heartbeats (short-term recordings). Generally, 5 minutes of data from 300 heartbeats to 400 heartbeats were recorded to evaluate the sympathetic and parasympathetic activities



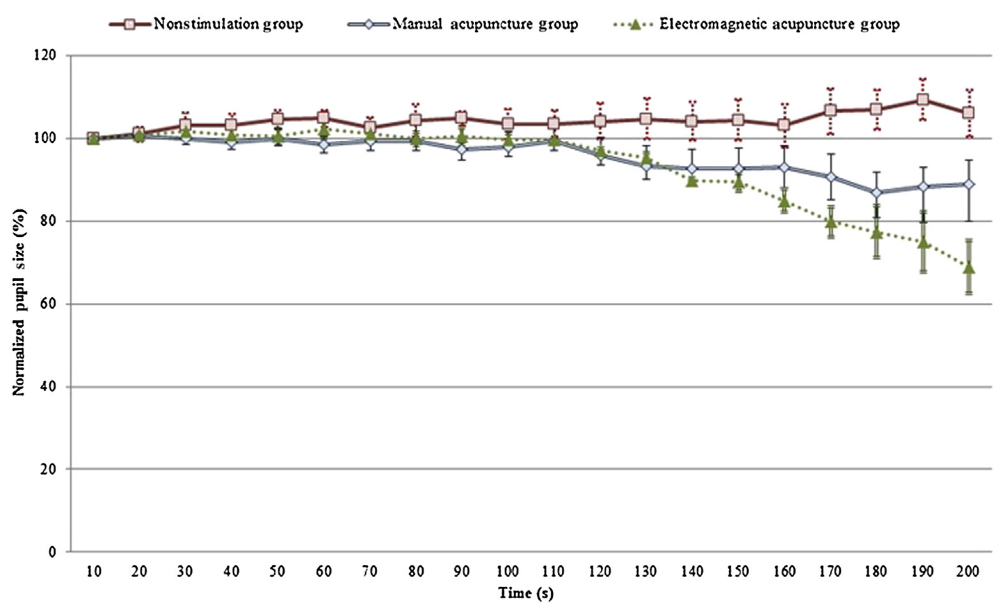


Figure 8 The mean and standard error of the pupil size for the three groups.

of the autonomic nervous system [39]. Although the HRV has emerged as a method of assessment to evaluate the sympathovagal balance, it still has several potential problems to solve. HRV is limited by positive predictive accuracy, sensitivity, and specificity. Also, it requires additional parameters such as gender, age, drug interferences, and concomitant diseases [40—42]. Moreover, it was reported that HRV has a significant relation with not only the activation of parasympathetic nerves, but also the autonomic nerve system dysfunction, correlation with obesity, and poor cardio respiratory fitness [43]. Although existing research selected and measured participants who do not have specific cardiovascular diseases, those experiments did not consider factors such as blood pressure and fat that may have an effect on HRV.

Because various factors affect HRV, we analyzed the pupil size to distinguish the activation of autonomic nerve system differently. As a result, we observed few changes in pupil size in the nonstimulation group for 200 seconds. The size of the pupil was 86.89 $\pm$ 15.22 (%) in the manual

Table 1 The significant probability analysis of the PSV in the nonstimulation group.

$n = 10$	After 20 s	After 40 s	After 60 s	After 80 s	After 100 s	After 120 s	After 140 s	After 160 s	After 180 s	After 200 s
p	0.607	0.319	0.077	0.326	0.402	0.380	0.420	0.583	0.218	0.341

PSV = pupil size variability.

Table 2 The significant probability analysis of the PSV in the manual acupuncture group.

n = 10	After 20 s	After 40 s	After 60 s	After 80 s	After 100 s	After 120 s	After 140 s	After 160 s	After 180 s	After 200 s
p	0.668	0.668	0.423	0.736	0.454	0.167	0.135	0.209	0.089	0.264

PSV = pupil size variability.

Table 3 The significant probability analysis of the PSV in the EMA group.

n = 10	After 20 s	After 40 s	After 60 s	After 80 s	After 100 s	After 120 s	After 140 s	After 160 s	After 180 s	After 200 s
p	0.872	0.497	0.782	0.383	0.292	0.119	0.031	0.010	0.014	0.010

EMA = electromagnetic acupuncture; PSV = pupil size variability.

acupuncture group compared with before stimulation. The greatest decline was observed in the EMA group, which had the pupil size of 71.04 $\pm$ 10.09 (%). However, there was no statistically significant difference in the manual acupuncture group as $p > 0.05$, and it was confirmed that only the EMA showed a significant difference after 140 seconds. Accordingly, it was proven that EMA has significant effects on the activation of parasympathetic nerves and the results regarding manual acupuncture was opposite to the results obtained in previous studies.

Additionally, as a result of measuring the biopotential to analyze the mechanism that affects the activation level of autonomic nerve systems, it was observed that biopotential was induced and median frequency (MF) had a significant difference only prior to and during stimulation ($p < 0.05$).

Skeletal muscle brings contraction phenomenon with the delivery of action potential to muscle fibers through the depolarization of α-neurons. Through nerve and muscle fibers, stimulation is created. Then, the ions in the muscles change. This change can be noticed with the EMG signal [44]. As the position of the electrodes is the same as the position of the electrode for measuring the electromyogram (EMG), biopotential $< \pm$ 20 uV means a stable state of muscle, when there is any activation inside the muscle, biopotential $> \pm$ 20 uV is created [44,45]. The median frequency (MF) during stimulation was higher than its level prior to stimulation. There was no significant difference between the median frequency (MF) prior to stimulation and its level after 120 seconds and 200 seconds. As shown in Figs. 9 and 10, the induced biopotential was composed of various frequency bands. Hence, the normal median frequency (MF) prior to stimulation significantly increased during stimulation. The increase in the median frequency (MF) indicates the increase in power in the frequency domain. Biopotential measured by the electrode position of the EMG signal is not generated voluntarily but by the stimulation of innervation. Because biopotential over 20 uV was created by EMA, it was considered that the ion inside

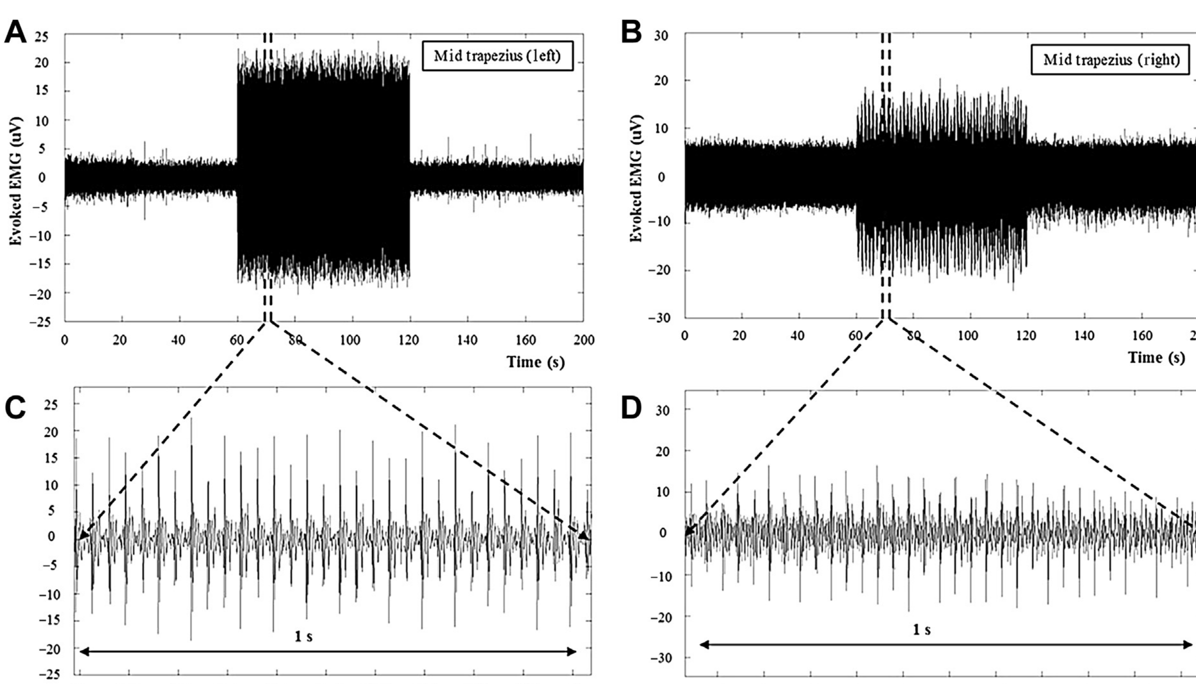


Figure 9 Biopotential at the left and right mid trapezius. EMG = electromyogram.

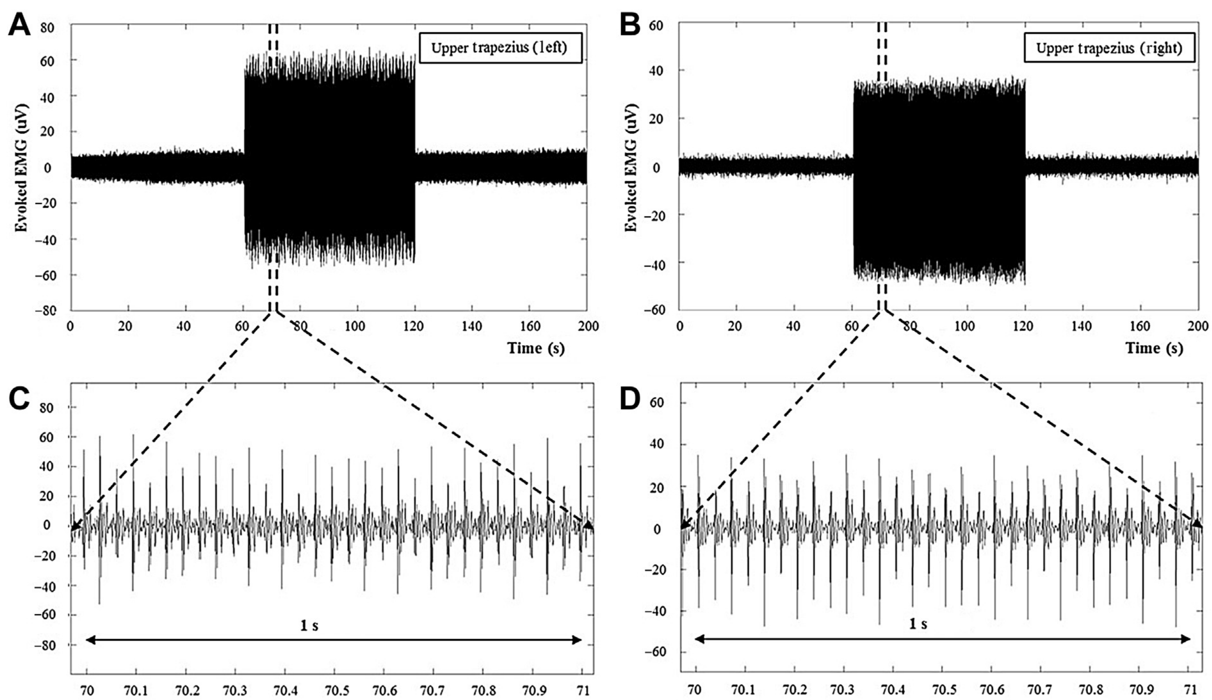


Figure 10 Biopotential at the left and right upper trapezius. EMG = electromyogram.

the muscle and connected nerve with related muscle changed by the generated action potential inside of the muscle incurred from EMA. Existing neurological research state that spinothalamic tract cells are hardly activated by an input on the skin creating stimulation in the heart and the lung. However, it is reported that they are stimulated significantly when the cells get additional input from muscles [25,26]. It was considered that activation of an autonomic nerve system decreases the size of pupils because EMA stimulates the acupoint and the muscle around the acupoint effectively.

To increase the therapeutic effect of manual acupuncture, we suggested simultaneous stimulation with manual acupuncture and weak magnetic fields. However, the weak magnetic fields have an insufficient energy transfer coefficient for acupoints in muscular tissue because of the geometrical decrease in magnetic flux density by distance. To solve this problem, this paper proposed a new method similar to EA which can create significant stimulation on acupoints by using the PEMFs, one of the weak magnetic fields.

To test the effect of an autonomic nervous system, this paper dealt with the changes in pupil size when stimulating at BL15 for three groups: nonstimulation group, manual acupuncture group, and EMA acupuncture group. In conclusion, we observed a greater decrease in the pupil size of the manual acupuncture group compared with the nonstimulation group; however, the difference was not statistically significant. In addition, the decrease in pupil size of the EMA group was observed in comparison to two groups. Through analyzing biopotential, we observed the biopotential generated inside the muscles. Based on the results, we recommended the EMA method. However, in the future it should be compared with EA. For further study, we

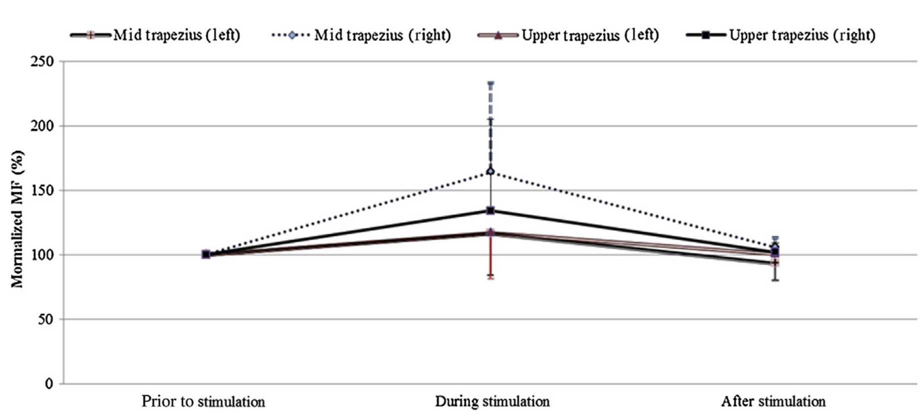


Figure 11 The mean and standard derivation of the normalized median frequency (MF).

can distinguish the best method to activate sympathetic nerves and parasympathetic nerves. Moreover, there is a need for further study to measure the changes in pupil size and HRV at the same time. Lastly, the biopotential needs to be analyzed when stimulating the EA or EMA. The generated biopotential is available as preliminary data to indicate the relationship between generated biopotential on an acupoint and the activation of the autonomic nerve system.

Disclosure statement

The author affirms there are no conflicts of interest and the author has no financial interest related to the material of this manuscript.

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